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# RESÚMENES

# Preliminary characterization of NADPH oxidase (NOX) activity in olive reproductive tissues

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NADPH oxidase (NOX) enzymes are a family of transmembrane proteins which transport electrons across the membrane from a cytosolic electron donor (NADPH in most of cases) to an extracellular electron acceptor. Available data suggest that the electron acceptor for plant NOX enzymes is oxygen and thus superoxide generation is the principal function of these enzymes, which are inhibited by diphenylene iodonium (DPI). Different plant NOX isoforms have been described, with apparent roles in plant defence functions as well as in growth and development [1, 2]. In plant reproductive tissues, the reactive oxygen species (ROS) derived from NADPH oxidase activity seem to be involved in generating or directing the polarized growth of *Nicotiana* pollen tubes [3]. The aim of this work was to determine whether NOX activity is present in the reproductive tissues of the olive (*Olea europaea* L.). Whole olive flowers or isolated pistils at different developmental stages were used to perform histochemical detection of superoxide with NBT, which forms a purple-brown precipitate (blue formazan) that localizes the site of superoxide production. Negative controls were treated the same, although they were preincubated with DPI. The presence of the precipitate was detected using stereomicroscopy, and images were captured with a digital camera. Similar experiments were carried out to detect general ROS production in whole flowers or isolated pistils by using the fluorescent indicator dye DCFH2-DA (2´,7´-dichlorodihydrofluorescein diacetate) either with or without DPI pre-incubation. The fluorescent signal was monitored by confocal laser scanning microscopy. Both procedures showed the presence of a DPI-sensitive signal in the olive stigma, whose intensity and distribution showed a precise developmental pattern. Mature pollen extracts were subjected to non-denaturing polyacrylamide gel electrophoresis (PAGE) with discontinuous buffer system in 7.5% (w/v) separating gel and 3% (w/v) stacking gel. Gels were stained for superoxide production by a modified NBT reduction method [4]. In the presence of NADPH, different superoxide-producing bands were identified. DPI was an efficient inhibitor of the formation of these bands. The comparison between pollen extracts from different olive cultivars showed differences in the number and the relative intensities of the corresponding bands. These results confirm the presence of NOX activity in the reproductive tissues of the olive. The physiological significance of this enzyme activity in the development of the floral organs and in the pollen-pistil interactions is discussed.

[1] Sagi and Fluhr (2006) *Plant Physiol.***141**:336-340
[2] K. Bedard *et al.* (2007) *Biochimie.* **89**:1107-1112
[3] Potocký *et al.* (2007) *New Phytol.* **174**:742-751
[4] Sagi and Fluhr (2001) *Plant Physiol.***126**:1281-1290

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